INTERACTIONS BETWEEN RESISTANCE-BREAKING *BEET NECROTIC YELLOW VEIN VIRUS* AND *BEET OAK-LEAF VIRUS* IN SUGAR BEET

Hsing-Yeh Liu* and Robert T. Lewellen
USDA-Agricultural Research Service, 1636 E. Alisal Street, Salinas, CA 93905

Abstract:

Rhizomania, a serious disease of sugar beet (*Beta vulgaris*), is caused by *Beet necrotic yellow vein virus* (BNYVV) and vectored by the plasmodiophorid *Polymyxa betae*. Resistance allele *Rz1* has been widely incorporated into commercial cultivars. Recently, resistance-breaking isolates of BNYVV (RB-BNYVV) were identified and characterized. When the occurrence of RB-BNYVV was surveyed throughout the sugar beet growing areas in the United States, most soil samples contained Beet oak-leaf virus (BOLV) as well. BNYVV and BOLV often occurred in the same field and sometimes in the same sugar beet plant. The possibility of interactions between these two *P. betae*-transmitted sugar beet viruses was tested. Plants grown in soils infested with aviruliferous *P. betae* or carrying RB-BNYVV and BOLV, alone and in combination, were compared with plants grown in non-infested soil for differences in plant fresh weight and virus content as measured by enzyme-linked immunosorbent assay (ELISA). *Rz1* and *Rz2* resistance genes that condition resistance to BNYVV did not confer resistance to BOLV. BNYVV ELISA values were significantly higher in single infections than in mixed infections with BOLV in both the rhizomania-resistant and -susceptible cultivars. In contrast, ELISA values of BOLV were not significantly different between single and mixed infections in both the rhizomania-resistant and -susceptible cultivars. Results indicate that BOLV may suppress BNYVV in mixed infections. Soils infested with *P. betae* significantly reduced fresh weight of sugar beet seedlings regardless of whether they were with or without one or both viruses or resistance genes.

Introduction:

Beet necrotic yellow vein virus (BNYVV) is a member of the genus *Benyvirus* (21, 22) and causes the disease known as rhizomania. It is often reported to be the most important sugar beet (*Beta vulgaris* L.) virus (17). BNYVV is transmitted by the plasmodiophorid, *Polymyxa betae* Keskin (1, 2, 6). In the United States, the virus was first identified in CA in 1984 (5) but now occurs in every major sugar beet production region in the country (17).

Rhizomania has caused major reductions in sugar beet root yield and sugar content. As soon as rhizomania was identified in North America, the USDA Agricultural Research Service in Salinas, CA began extensively screening genetic resources to identify potential sources of resistance to BNYVV and incorporate resistance into sugar beet germplasm (3, 10). *Rz1* is a single dominant resistant gene for BNYVV (12) and is the only major resistance gene identified within sugar beet germplasm (3, 20). A second resistance gene, derived from wild beet (WB42) (9) designated as *Rz2* (18, 19), was shown to be different from *Rz1* and conferred a higher level of resistance (16, 19). Recently, a third resistance gene *Rz3* has been reported from WB41 (9), which is linked to *Rz1* and *Rz2* on chromosome III (8). Plants with combined *Rz1* and *Rz2* or *Rz3*

in a heterozygous condition have lower titer for BNYVV than with *Rz1* alone (15). At the present time, planting partial resistant cultivars are the only economical way to control this devastating disease.

In 2002-2003, severe symptoms of rhizomania were observed in the rhizomania-resistant *Rz1* sugar beet cultivars planted in the Imperial Valley of California. We soon verified that certain isolates of BNYVV from the Imperial Valley had overcome *Rz1* resistance gene (15). Since 2003, not only did the resistance-breaking BNYVV (RB-BNYVV) appear in the Imperial Valley, but also in other sugar beet growing regions in the United States including CO, ID, MN, NE, and OR (13).

During the survey for RB-BNYVV isolates in the United States, Beet oak-leaf virus (BOLV) (14) was frequently found co-infected with BNYVV in the same field and sometimes in the same sugar beet plant. BOLV was first isolated from rhizomania infested fields in California. Infected sugar beet leaves showed oak-leaf pattern symptoms different from rhizomania. BOLV is serologically distinct from BNYVV, *Beet soil-borne mosaic benyvirus* (BSBMV), and *Beet soil-borne pomovirus* (BSBV) (14). The host range of BOLV, which is largely confined to Chenopodiaceous plants, is similar to BNYVV and BSBMV. BOLV has been purified from spinach (*Spinacia oleracea*). Virus particles are 20 nm wide and ranged from 80 to 640 nm in lengths. BOLV is transmitted also by *P. betae*. The molecular mass of the capsid protein was estimated as 46.0 kDa. The taxonomic status of BOLV has not yet been determined. A polyclonal antibody from rabbits has been produced against BOLV and can be used in ELISA, western blot, and immunogold labeling tests. BOLV appears to be spread widely in the U.S. It has been found in CA, CO, MI, MN, NE, and WY. In contrast to BNYVV, little is known about the effect of BOLV on yield and sugar content in sugar beet.

The objectives of this study were to determine if the *Rz* BNYVV resistance genes confer resistance to BOLV and to determine the effects of *P. betae*, RB-BNYVV isolates and BOLV alone and in combination, on growth and relative ELISA values in sugar beet.

Materials and Methods:

Inoculum preparation. RB-BNYVV isolates were collected from the Imperial Valley, CA; BOLV isolates were collected from Salinas, CA. BNYVV and BOLV isolates were mechanically inoculated to the systemic hosts *Beta macrocarpa* or spinach (*S. oleracea*), which were planted in sterilized soil. After the plants exhibited symptoms of systemic infection, virus-free *P. betae* was incorporated into the soil of infected plants by mixing with water. One month later, infected roots and soil were harvested and used for inocula for further experiments. The aviruliferous *P. betae* was obtained from river sand collected from eastern WY. Roots from seedlings grown in soil containing this *P. betae* isolate were tested repeatedly by enzyme-linked immunosorbet assay (ELISA) for the presence of these two soil-borne viruses to assure that no viruses were present. All virus-infested soils and virus-free soils were tested by baited plant techniques prior to these studies to confirm the presence or absence of the desired viruses and *P. betae* (15).

Greenhouse experiments. Four hybrid sugar beet cultivars were used in greenhouse experiments. Beta4430R (*Rz1rz1*), BetaG017R (*Rz2rz2*), and KWS Angelina (*Rz1rz1* + *Rz2rz2*) are partially resistant to BNYVV. Triploid Beta6600 (*rz1rz1rz1*) was used as the susceptible check. Greenhouse benches were washed in 10% sodium hypochlorite prior to use. One part of each of the stock infested soil samples was mixed with nine parts of autoclaved builder's sand

for inoculum. The soils were then filled into pots (new 280-ml Styrofoam cups) arranged on greenhouse benches in a completely random design with three replications for each treatment. The greenhouse was maintained between 15 and 24°C without supplemental light. In experiments I and II, plants of each sugar beet cultivar were subject to five soil treatments: (i) sterilized soil; (ii) aviruliferous *P. betae*-infested soil; (iii) RB-BNYVV-infested soil; (iv) BOLV-infested soil; and (v) RB-BNYVV- and BOLV-infested soil, mixed in equal parts. One hundred seeds of the tested cultivars were directly sown in each pot. At 4, 6, 8, and 10 weeks post-emergence, seedlings and roots from these pots were harvested. The seedlings in each pot were weighted immediately after washing and removing water on the seedling with paper towels. The roots were tested for relative concentration of BNYVV and BOLV by ELISA as described below. In experiment III, soil treatments were modified by sequentially transplanting sugar beet seedlings of each cultivar into one soil for a month then washing to remove the soil and re-transplanting into another soil until harvest. Three soils (Healthy=Sterilized virus free soil, BNYVV=soil infested with RB-BNYVV, BOLV=soil infested with BOLV) are combined into seven soil treatments: (i) Healthy/Healthy (ii) Healthy/BNYVV; (iii) Healthy/BOL); (iv) BNYVV/Healthy; (v) BOLV/Healthy; (vi) BNYVV/BOLV; and (vii) BOLV/BNYVV. Plants were harvested 6 weeks after the second transplanting. The roots were washed and tested for relative concentration of BNYVV and BOLV by ELISA as described below. Treatments were arranged in a completely random design with three replications for each treatment in experiments I and II, and six replications in experiment III.

Enzyme-linked immunosorbent assay (ELISA). Roots samples collected from each pot were washed with tap water to remove soil. Root tissue weighing 0.2 g was taken from each root mass, placed in sample extraction bags containing 2 ml of extraction buffer (0.05 M phosphate-buffered saline, pH 7.2, 0.5% Tween-20, 0.4% dry milk powder) and homogenized with a hand-held roller press (Agdia, Inc., Elkhart, IN). Expressed sap (100 µl per well) was added to duplicate wells of a microtiter plate. Each plate also contained controls including sap from BNYVV-infected beet roots, BOLV-infected beet roots, and healthy beet roots.

Double antibody sandwich ELISA (4) was used. Purified IgG made to BNYVV and BOLV (1mg/ml) were used to coat microtiter plates at a 1/1000 dilution. Alkaline phosphatase-conjugated anti-BNYVV/BOLV IgG was added to wells (1/1000 dilution). Alkaline phosphatase substrate (Sigma Chemical, St. Louis, MO) was used at a ratio of 5 mg/8.3 ml of substrate buffer. Absorbance readings (A_{405nm}) were made 1 hr after the addition of substrate using a Bio-Tek EL312e microplate reader (Winooski, VT).

Data analysis. Analysis of variance was performed to test the effects of soil treatments, sugar beet cultivars, and harvest time, as well as their interactions on the average fresh weight per seedling at harvest for experiments I and II, and on the relative ELISA value for all experiments using general linear model (GLM procedure) in SAS (Rev 9.1.3 SAS Institute Inc., Cary, NC 27513). Multiple range tests were conducted to compare treatment means whenever the treatment effects were significant.

Results:

Experiments I and II. Analysis of variance demonstrated that cultivar, soil treatments, harvest date and interaction of cultivar × harvest date all affected seedling weight significantly (Table 1). There was a significant effect of sugar beet cultivar on BNYVV titers, but not on BOLV titers (Table 1). There were significant effects of soil treatments and harvest times on ELISA values of both viruses (Table 1). There was a significant effect of soil treatment × harvest time interaction for ELISA values for both viruses (Table 1). An effect of interactions of soil treatment × cultivar, and cultivar × harvest date was either inconsistent between two experiments or insignificant (Table 1). ELISA values of BNYVV for Beta6600 and Beta4430R were significantly higher than for cultivars Beta G017R and KWS Angelina (data not shown), but titers for all cultivars were significantly higher for BNYVV when the seedlings were grown in BNYVV-infested soil than when they were grown in the sterilized soil, indicating moderate resistance against the RB-BNYVV isolates in cultivars with the *Rz2* gene (data not shown). Resistance was not observed in any cultivar tested against BOLV. Seedlings grown in the BOLV-infested soil showed significantly higher ELISA values of BOLV than seedlings grown in the sterilized soil. ELISA values of BNYVV were consistently lower in soil infested by BNYVV+BOLV, than by BNYVV alone and both soils had significantly higher titers of BNYVV than in soils not infested or infested with BOLV alone (Table 2). Titers for BOLV were not consistently different between treatments for BOLV and BOLV+BNYVV, but both were significantly higher than any other treatment (Table 2). **V**irus-free *P. betae* caused a significant reduction in average seedling weight on two sugar beet cultivars in experiment I and on all four tested cultivars in experiment II (Table 3). The greatest reduction in growth was for 'Beta4430R' which has *Rz1* resistant gene and 'KWS Angelina' which has both *Rz1* and *Rz2* resistant genes. Under microscopic examination involving many tests over several years, roots from Beta4430R and KWS Angelina consistently had higher counts of *P. betae* cystosori than most other cultivars with or without *Rz1* examined (Liu, unpublished data). There was no significant difference in average seedling weight among treatments in which *P. betae* was infested with BNYVV and/or BOLV alone or in combination (data not shown).

Table 1. Analyses of variance for enzyme-linked immunosorbent assay value for *Beet necrotic yellow vein virus* (BNYVV) and Beet oak-leaf virus (BOLV), and average fresh weight per plant for four cultivars grown under 5 soil treatments and 4 harvest dates.

		Mean squares [u]					
		Experiment I			**Experiment II**		
Source	**df**	**BNYVV** [v]	**BOLV** [v]	**Weight (g)** [w]	**BNYVV**	**BOLV**	**Weight (g)**
Cultivar (C) [x]	3	3.294 **	2.057 ns	0.127 **	15.674 **	2.810 ns	0.121 **
Soil Treatment (ST) [y]	4	197.709 **	86.076 **	0.013 **	295.477 **	819.418 **	0.364 **
C X ST	12	0.794 ns	1.468 ns	0.002 ns	7.074 **	3.230 *	0.006 ns
Harvest Date (HD) [z]	3	11.593 **	5.587 **	0.018 **	34.650 **	107.928 **	0.240 **
C X HD	9	4.359 ns	0.548 ns	0.006 *	1.385 ns	1.034 ns	0.012 *
ST X HD	12	3.315 **	2.608 *	0.001 ns	12.503 **	38.894 **	0.006 ns
C X ST X HD	36	14.948 ns	0.693 ns	0.003 ns	1.586 *	1.459 ns	0.007 ns
Error	158	90.397	1.267	0.002	0.924	1.739	0.006

[u] Experiments I and II: completely random design with three repetitions; ns = not significant; * and ** indicate significance at the $P \leq 0.05$ and 0.01 levels, respectively, according to the *F* test.

[v] Values represent the ratio of the absorbance at 405 nm reading for BNYVV or BOLV over the corresponding healthy absorbance value.

[w] Average fresh weight per plant.

[x] Rhizomania susceptible cultivar without known resistant gene: Beta 6600, rhizomania resistant cultivars: Beta4430R, BetaG017R, and KWS Angelina with *Rz1, Rz2*, and *Rz1+Rz2* resistant genes, respectively.

[y] Sterilized soil, soil with non-viruliferous *Polymyxa betae*, BNYVV viruliferous *P. betae*, BOLV viruliferous *P. betae*, and BNYVV and BOLV viruliferous *P. betae*.

[z] 4, 6, 8, and 10 weeks post emergence.

Experiment III. Analysis of variance revealed significant effects for cultivars, first soil treatment, second soil treatment and all interactions among cultivar and soil treatment (Table 4). Seedlings grown first in BNYVV-infested soil and those transferred into BNYVV-infested soil all had significantly higher BNYVV titers than the seedlings retained in the sterilized soil or transferred from or into soil infested with BOLV. Based upon ELISA values, differences exhibited among cultivars were insignificant, suggesting no resistance against RB-BNYVV isolates in any of the four cultivars tested (Table 5). Resistance was not observed against BOLV in the four cultivars in terms of BOLV titers (Table 5). Sugar beet seedlings of both resistant and susceptible cultivars initially grown in BOLV infested soil then transferred into BNYVV-infested soil or initially grown in BNYVV-infested soil then transferred into BOLV infested soil had significantly reduced BNYVV titers compared to seedlings transferred from healthy soil to BNYVV-infested soil, and from BNYVV-infested soil to healthy soil (Table 5). The ELISA values for BOLV either were not significantly different or were significantly higher in mixed infection than when infected with BOLV alone (Table 5).

Discussion:

BOLV is observed widely in sugar beet in the U.S. (14). BOLV and BNYVV share the same vector, *P. betae*, have similar biological characteristics, and often are found in the same field and sugar beet plant. Resistance genes *Rz1* and *Rz2* condition host-plant reaction to some isolates of BNYVV but their effect on BOLV was unknown. Interactions between BOLV and BNYVV in dual infections were also unknown. In this research we investigated the effects of RB-BNYVV or BOLV infection alone and RB-BNYVV and BOLV mixed infections on relative virus levels by ELISA tests in greenhouse pot culture. The use of ELISA for detection of BNYVV is highly reliable and has been accepted as the sugar beet industry standard for

Table 2. Enzyme-linked immunosorbent assay values for *Beet necrotic yellow vein virus* (BNYVV) and Beet oak-leaf virus (BOLV) in root tissue grown under 5 soil treatments with or without one or both viruses.

	Experiment I		Experiment II	
Treatments	**BNYVV** [z]	**BOLV** [z]	**BNYVV**	**BOLV**
Noninfested	1.001 d	0.978 c	1.018 c	1.018 c
Polymyxa betae	1.784 c	1.220 c	1.051 c	1.133 c
BNYVV	5.439 a	1.155 c	6.272 a	1.343 c
BOLV	1.111 d	3.786 a	1.132 c	8.291 b
BNYVV + BOLV	4.352 b	3.286 b	4.671 b	9.084 a

[z] Values represent the ratio of the absorbance at 405 nm reading for BNYVV or BOLV over the corresponding healthy absorbance value. Means within columns followed by a different letter are significant at $P \leq 0.05$ according to the Duncan's multiple range test.

Table 3. Effects of aviruliferous *Polymyxa betae*-infested soil on plant weight in rhizomania-susceptible and-resistant sugar beet cultivars [y]

Sugar beet cultivar [z]	**Average weight per plant (g)**			
	Experiment I		**Experiment II**	
	Non-infested soil	***Polymyxa betae* infested soil**	**Non-infested soil**	***Polymyxa betae* infested soil**
Beta6600	0.339 a	0.311 a	0.475 a	0.349 b
Beta4430R	0.252 a	0.200 b	0.508 a	0.350 b
BetaG017R	0.232 a	0.211 a	0.463 a	0.298 b
KWS Angelina	0.289 a	0.227 b	0.442 a	0.229 b

[y] Means of 12 pots across four harvest dates and three repetitions. Means within rows of each test followed by a different letter are significant at $P \leq 0.05$ according to the Duncan's multiple range test.

[z] Beta6600 is susceptible to rhizomania without known resistance gene; Beta4430R, BetaG017R, and KWS Angelina are resistanct cultivars to rhizomania with *Rz1, Rz2*, and *Rz1+Rz2* resistance genes, respectively.

Table 4. Analyses of variance for enzyme-linked immunosorbent assay value for *Beet necrotic yellow vein virus* (BNYVV) and Beet oak-leaf virus (BOLV) on interactions of sugar beet cultivars [y] and soil treatments [z].

Source	**df**	**Type III sum square**	***F* value**	***Pr. > F***
BNYVV				
Cultivar (C)	3	21.6	6.4	0.0004
1st treatment (1st T)	2	302.5	134.2	< 0.0001
C x 1st T	6	17.6	2.6	0.0202
2nd treatment (2nd T)	2	442.1	196.2	< 0.0001
C x 2nd T	6	23.1	3.4	0.0035
1st T x 2nd T	2	30.8	13.7	< 0.0001
C x 1st T x 2nd T	6	15.6	2.3	0.0371
Error	134	1.12		
BOLV				
Cultivar (C)	3	11.9	4.5	0.0050
1st treatment (1st T)	2	362.8	204.8	< 0.0001
C x 1st T	6	14.0	2.6	0.0191
2nd treatment (2nd T)	2	162.3	91.6	< 0.0001
C x 2nd T	6	19.1	3.6	0.0024
1st T x 2nd T	2	29.8	16.8	< 0.0001
C x 1st T x 2nd T	6	16.3	3.1	0.0076
Error	134	0.88		

[y] Four sugar beet cultivars used are: Beta6600 without known resistance gene against rhizomania; Beta4430R, BetaG017R, and KWS Angelina with *Rz1*, *Rz2*, and *Rz1+Rz2* resistance genes, respectively.

[z] Soil treatments: sequentially transplanting sugar beet seedlings of each cultivar into one soil for a month (1st treatment) then washing to remove the soil and re-transplanting into another soil (2nd treatment) until harvest. Three soils (Healthy: Sterilized virus free soil; BNYVV: infested with RB-BNYVV, and BOLV: infested with BOLV) are combined into seven soil treatments: (i) Healthy/Healthy (ii) Healthy/BNYVV; (iii) Healthy/BOL); (iv) BNYVV/Healthy; (v) BOLV/Healthy; (vi) BNYVV/BOLV; and (vii) BOLV/BNYVV.

Table 5. Enzyme-linked immunosorbent assay value interaction means among cultivars and challenge test for *Beet necrotic yellow vein virus* (BNYVV) and Beet oak-leaf virus (BOLV).

Cultivar [x] **and Soil Treatment** [y]	**BNYVV** [z]	**BOLV** [z]
Beta6600-Healthy/Healthy [z]	0.977c	0.957c
-Healthy/BNYVV	5.736a	1.064c
-Healthy/BOLV	1.118c	3.348b
-BNYVV/Healthy	5.170a	1.198c
-BOLV/ Healthy	1.021c	3.520b
-BNYVV/BOLV	3.453b	4.107b
-BOLV/BNYVV	5.715a	7.155a
Beta4430R-Healthy/Healthy	0.984b	1.017c
-Healthy/BNYVV	5.849a	1.180c
-Healthy/BOLV	1.160b	2.218b
-BNYVV/Healthy	4.917a	1.146c
-BOLV/ Healthy	1.166b	3.436b
-BNYVV/BOLV	4.783a	4.332b
-BOLV/BNYVV	4.951a	6.773a
BetaG017R-Healthy/Healthy	1.038c	1.028c
-Healthy/BNYVV	5.986a	0.998c
-Healthy/BOLV	1.105c	3.336b
-BNYVV/Healthy	5.722a	1.168c
-BOLV/ Healthy	1.062c	4.155a
-BNYVV/BOLV	2.207b	2.757b
-BOLV/BNYVV	2.560b	4.172a
KWS-Angelina-Healthy/Healthy	0.990c	1.010c
-Healthy/BNYVV	5.128a	1.124c
-Healthy/BOLV	1.266c	3.287b
-BNYVV/Healthy	4.736a	1.164c
-BOLV/ Healthy	1.150c	3.730b
-BNYVV/BOLV	2.708b	3.168b
-BOLV/BNYVV	3.346b	5.822a

[x] Beta6600 without known resistance gene against rhizomania; Beta4430R, BetaG017R, and KWS Angelina with *Rz1, Rz2*, and *Rz1+Rz2* resistance genes, respectively.

[y] Sugar beet seedling grown in 1st soil treatment for four weeks/transfer to 2nd soil treatment for six weeks before harvest.

[z] Values represent the ratio of the absorbance at 405 nm reading for BNYVV or BOLV over the corresponding healthy absorbance value. Means within cultivar followed by a different letter are significant at $P \leq 0.05$ according to the Duncan's multiple range test.

confirmation of rhizomania-infested fields since the late 1980s (7, 23, 25). Specific antisera developed at the USDA-ARS in Salinas, CA were highly selective in differentiating BNYVV or BOLV infection. Root samples from non-infested soil and from aviruliferous *P. betae* soil treatments were negative to both BNYVV and BOLV in ELISA tests (Table 2). These negative scores indicated that there was no contamination with either virus in the virus-free *P. betae* population or in the non-infested soil. In BNYVV or BOLV soil, baited plant root samples were

only positive to BNYVV or BOLV, which proved that these soil samples contained only one virus and were not contaminated with the other virus (Table 2).

The effects of mixed and sequential infections of BNYVV and BOLV were examined in four varieties in three experiments, which were conducted in different seasons. Similar effects were observed despite different growth rates between seasons. Relative virus levels appeared to be fundamental to the plant host interaction rather than to plant growth or seasonal influences.

RB-BNYVV ELISA values were significantly higher in the susceptible cultivar (Beta6600) and the cultivar with the *Rz1* resistant gene (Beta4430R) than cultivars containing *Rz2* or *Rz1* and *Rz2* resistance genes (BetaG017 R and KWS Angelina) (data not shown). These results coincided with our soil survey results (13). Cultivars with the *Rz1* resistant gene have been used in fields since 1988 and RB-BNYVV isolates that might overcome resistance from the *Rz1* allele could have been selected.

Previously it was known that BNYVV and BOLV are serologically distinct (14). Based on the similar ELISA values for BOLV in all four resistance gene combinations (Table 5), it appears that the *Rz* genes do not confer resistance or differential responses to BOLV infection. This lack of response to the *Rz* genotypes further demonstrates that BOLV and BNYVV are distinct.

Reductions in seedling weight occurred when sugar beet seedlings were grown in *P. betae* infested soil compared with non-infested soil. A significant reduction occurred in seedling weight when BNYVV resistant cultivars Beta4430R and KWS Angelina were grown in *P. betae* infested soil. This outcome revealed that *Rz1* and *Rz2* resistant genes condition resistance to BNYVV but not to *P. betae*. Based upon many microscopic examinations across many cultivars and genotypes, it is believed that the high *P. betae* cystosori counts in Beta4430R and Angelina are coincidental and are likely due to the background genotypes of these two cultivars independent of the occurrence of *Rz1* or *Rz2*.

The relationship between competing infectious agents in the same host is not well understood but can sometimes be exploited to help mitigate damage caused by the more severe agent. In this research, when RB-BNYVV existed in mixed infections with BOLV, the level of RB-BNYVV was significantly reduced in all cultivars tested. However, the level of BOLV remained the same in single infections or in mixed infections with BNYVV (Table 2). Previously, another *P. betae*-vectored virus of sugar beet, *Beet soil-borne mosaic virus*, suppressed BNYVV in dual infections (24). The mechanism for the suppression of BNYVV by these viruses is not known.

Rhizomania is one of the most devastating diseases of sugar beet (11). Without the use of cultivars with *Rz1* or *Rz2*-mediated resistance to BNYVV, much of the sugar beet production worldwide could cease. The appearance of resistance-breaking isolates of BNYVV again puts crops at risk. Soil fumigation can be little used because of regulatory, environmental, and economic issues. Protective chemicals are not available against *P. betae*. Beyond the deployment of resistant genes, alternative control measures should be considered, sought, and evaluated. If BOLV causes little or no damage on sugar beet production, it may be useful to consider BOLV as a potential suppressive agent in heavily rhizomania (BNYVV)-infested fields. In rhizomania evaluation nurseries at Salinas, CA that contained both BNYVV and BOLV, BNYVV titer and root symptoms in several sequential sugar beet crops appeared to be suppressed (Lewellen & Liu, unpublished data). This suppressive entity is not known, but these results would suggest that it may be BOLV.

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Literature Cited:

1. Abe, H., and Tamada, T. 1986. Association of *Beet necrotic yellow vein virus* with isolates of *Polymyxa betae* Keskin. Ann. Phytopathol. Soc. Jpn. 52:235-247.
2. Barr, D. J. S. 1992. Evolution and kingdoms of organisms from the perspective of a mycologist. Mycologia 84:1-11.
3. Biancardi, E., Lewellen, R. T., Biaggi, M. D., Erichsen, A. W., and Stevanato, P. 2002. The origin of rhizomania resistance in sugarbeet. Euphytica 127:383-397.
4. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
5. Duffus, J. E., Whitney, E. D., Larson, R. C., Liu, H.-Y., and Lewellen, R. T. 1984. First report in Western hemisphere of rhizomania of sugar beet caused by *Beet necrotic yellow vein virus*. Plant Dis. 68:251.
6. Fujisawa, I. and Sugimoto, T. 1976. Transmission of *Beet necrotic yellow vein virus* by *Polymyxa betae.* Ann. Phytopathol. Soc. Japan. 43:583-586.
7. Gerik, J. S., Jensen, C. S., and Duffus, J. E. 1987. Soil testing for Rhizomania. Calif. Sugar Beet 1987:17-28.
8. Gidner, S., Lennefors, B.-L., Nilsson, N.-O., Bensefelt, J., Johansson, E., Gyllenspetz, U., and Kraft, T. 2005. QTL mapping of BNYVV resistance from the WB41 source of sugar beet. Genome 48:279-285.
9. Lewellen, R. T. 1997. Registration of 11 sugarbeet germplasm lines with resistance to rhizomania. Crop Sci. 37:1026.
10. Lewellen, R. T. 2000. Registration of rhizomania resistant sugarbeet x Beta vulgaris subsp. maritima germplasms C26, C27, and C51. Crop Sci. 40:1513-11515.
11. Lewellen, R. T. and Biancardi, E. 1990. Breeding and performance of rhizomania tolerant sugar beet. Pages 79-84 in: Proc. 53rd Winter Congress, I.I.R.B., Brussels, Belgium.
12. Lewellen, R. T., Skoyen, I. O., and Erichsen, A. W. 1987. Breeding sugarbeet for resistance to rhizomania: evaluation of host-plant reactions and selection for and inheritance of resistance. In: Proceedings of the 50th Winter Congress of the IIRB, 1987. International Institute for Beet Research, Brussels, pp 139-156.
13. Liu, H.-Y. and Lewellen, R. T. 2007. Distribution and molecular characterization of resistance-breaking isolates of *Beet necrotic yellow vein virus* in the United States. Plant Dis. 91:847-851.
14. Liu, H.-Y., Sears, J. L., and Lewellen, R. T. 2003. Beet oak-leaf virus – a new *Polymyxa betae* transmitted sugar beet virus in the United States. In: Proceedings of 8th International Congress of Plant Pathology. p268. Christchurch, New Zealand, February 2-7, 2003.
15. Liu, H.-Y., Sears, J. L., and Lewellen, R. T. 2005. Occurrence of resistance-breaking *Beet necrotic yellow vein virus* of sugar beet. Plant Dis. 89:464-468.

16. Panella, L. and Lewellen, R. T. 2007. Broadening the genetic base of sugar beet: introgression from wild relatives. Euphytica 154:383-400.
17. Rush, C. M., Liu, H.-Y., Lewellen, R. T., and Acosta-Leal, R. 2006. The continuing saga of rhizomania of sugar beets in the United States. Plant Dis 90:4-15.
18. Scholten, O. E., DeBock, T. S. M., Klein-Lankhorst, R. M., and Lange, W. 1999. Inheritance of resistance to *Beet necrotic yellow vein virus* in *Beta vulgaris* conferred by a second gene for resistance. Theor Appl. Genet 99:740-746.
19. Scholten, O. E., Jansen, R. C., Keiser, L. C. P., DeBock, T. S. M., and Lange, W. 1996. Major genes for resistance to *Beet necrotic yellow vein virus* (BNYVV) in *Beta vulgaris*. Euphytica 91:331-339.
20. Scholten, O. E. and Lange W. 2000. Breeding for resistance to rhizomania in sugar beet: a review. Euphytica 112:219-231.
21. Tamada, T. 1999. *Benyviruses*. Pages 154-160 in: Encyclopedia of Virology. R. G. Webster and A. Granoff, eds. Academic Press, London.
22. Torrance L., and Mayo, M. A. 1997. Proposed re-classification of *Furoviruses*. Arch. Virol. 142:435-439.
23. Wisler, G. C., Lewellen, R. T., Sears, J. L., Liu, H. Y., and Duffus, J. E. 1999. Specificity of TSA-ELISA for *Beet necrotic yellow vein virus* and its application for determining rhizomania resistance in field-grown sugar beets. Plant Dis. 83:864-870.
24. Wisler, G. C., Lewellen, R. T., Sears, J. L., Wasson, J. W., Liu, H. Y., and Wintermantel, W. M. 2003. Interactions between *Beet necrotic yellow vein virus* and *Beet soilborne mosaic virus* in sugar beet. Plant Dis. 87:1170-1175.
25. Wisler, G. C., Liu, H. Y., and Duffus, J. E. 1994. Beet necrotic yellow vein virus and its relationship to eight sugar beet furo-like viruses from the United States. Plant Dis. 78:995-1001.